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In vitro Lymphocyte Reactivity during Depression of Tuberculin Hypersensitivity by 6-Mercaptopurine

Abstract. *Administration of 6-mercaptopurine suppressed appearance of tuberculin skin test reactivity for up to 6 weeks after mycobacterial injection. Lymphocytes obtained during the period of suppressed tuberculin reactivity exhibited normal in vitro proliferative responses to tuberculin, suggesting that the drug may not be qualitatively affecting function of immunologically competent cells.*

Several studies (*1*, *2*) have demonstrated a suppressive effect of 6-mercaptopurine and its analogs on delayed hypersensitivity. The present study was undertaken to further elucidate the cellular mechanisms in this suppressive effect. In a previous study in our laboratory (*3*) we showed that in guinea pigs sensitized with mycobacteria, tuberculin skin test reactivity and in vitro proliferative responsiveness to tuberculin by peripheral blood lymphocytes develop concomitantly. In the present work we investigated the effect of 6-mercaptopurine treatment on the development of these reactivities in guinea pigs similarly injected with mycobacteria.

Male albino guinea pigs, 500 to 700 g, were sensitized by footpad and subcutaneous injections of 1.0 ml of complete Freund's adjuvant containing 1.0 mg of heat-killed H-37 Ra mycobacteria per milliliter. From the 4th to the 14th day after the injection, each animal received either (i) 6-mercaptopurine in daily intramuscular injections of 50 mg/kg or (ii) an equal volume of control solution of 1.0*N* NaOH, which had been used as the diluent for the 6-mercaptopurine. Each animal was skin tested at weekly intervals from the day before sensitization until termination of the study. Agents used for skin testing were PPD-S, 0.1 μg/0.1 ml (5 tuberculin units), and old tuberculin, 0.01 mg/0.1 ml (Koch's Old Tuberculin). Skin tests were expressed as the mean of two perpendicular diameters of induration at 24-hour readings.

Immediately before sensitization and at weekly intervals thereafter, sterile heparinized blood was obtained by cardiac puncture and sedimented by using the method of Hulliger and Blazkovec (*4*). The leukocyte-rich supernatant layer was centrifuged; the resultant cell button was washed twice with Hanks solution, and resuspended in a culture medium containing Eagle's minimal essential medium, 20 percent serum from newborn pre-colostrum calves (Colorado Serum Co.), glutamine (final concentration, 2 percent), and penicillin and streptomycin (final concentration, 100 units and 100 μg/ml, respectively). Each culture vial contained 1×10^6 lymphocytes in a total volume of 1.0 ml. To selected test vials were added 0.5 μg of Koch's Old Tuberculin for comparison with control vials containing no antigen. Replicate cultures were incubated at 37°C in CO_2–air (5 : 95) for 5 days. Proliferative responses of the lymphocytes were measured by incorporation of H^3-thymidine (specific activity, 6.7 c/mmole; New England Nuclear Co.) into DNA as previously described (*5*). The response was expressed as an Isotope Incorporation Index (I.I.I.) equal to the ratio of the mean counts per minute of replicate test vials to the mean counts per minute in replicate control vials for any particular experiment. In selected experiments, cytologic studies looking for blast cell transformation and mitotic division were carried out as described previously (*6*).

Some of the guinea pigs not treated with 6-mercaptopurine showed positive skin test and in vitro lymphocyte proliferative (I.I.I. > 3) reactivities to tuberculin starting on day 14 after sensitization. These positive responses were present to varying degrees in almost all these animals by day 21 (Table 1). The in vitro antigen-induced proliferative response was shown not only by increasing isotope uptake (I.I.I.) but by increased blast cell transformation and mitotic division in lymphocytes cultured with tuberculin.

Guinea pigs treated with 6-mercapto-

Table 1. Skin test and lymphocyte reactivities in control group on day 21 after complete Freund's adjuvant. Skin test = PPD skin test reactivity (mean diameter of induration at 24 hours). I.I.I. = lymphocyte proliferative response (see text). Percentage of blasts = blast cells/total lymphocytes, at time of harvest, × 100. M.I. (mitotic index) = cells in mitoses/1000 lymphocytes at time of harvest.

Animal No.	Skin test	I.I.I.	Blood lymphocytes per mm³	Percentage of blasts/ M.I.
12	7	4.4	6255	31/8
16	8	3.2	6930	31/6
18	8	29.4	4900	41/24
22	10	1.9	5495	20/4
23	6	6.4	5775	42/9
28	6	24.2	4800	39/21
29	6	22.8	5100	40/21
46	11	30.4	9500	55/24
47	12	10.4	6000	24/12
52	6	5.0	2500	
53	7	21.0	3000	
67	6	31.5	3400	
68	10	1.5	3100	
75	6	3.2	2000	
76	5	1.5	4700	
Means ±S.E.	7.6± 2.1	12.4± 10.6	4963± 1701	38±9.1/ 14±7.7

Table 2. Skin test and lymphocyte reactivities in 6-mercaptopurine group on day 21 after complete Freund's adjuvant.

Animal No.	Skin test	I.I.I.	Blood lymphocytes per mm³	Percentage of blasts/ M.I.
17	0	4.3	1850	20/5
19	2	6.5	2000	31/11
21	2	2.6	2145	25/6
24	3	11.5	2700	54/18
25	1	16.0	2775	72/15
31	1	15.5	4100	30/12
32	2	42.1	2100	40/36
42	0	1.0	950	15/0
43	0	5.5	1560	38/10
44	2	4.0	1000	28/5
45	0	6.0	2100	35/9
49	3	16.0	2400	
51	2	4.1	2600	
61	1	16.0	4900	
62	0	2.8	3900	
64	1	38.0	3600	
65	1	37.0	2500	
66	2	4.5	2800	
71	1	3.1	1640	
72	0	3.0	1940	
74	1	7.0	2130	
Means S.E.±	1.2± 0.94	11.7± 3.18	2490± 985	35±15/ 13±9

purine generally demonstrated little or no tubercu'in skin reactivity on day 21 after injection of the mycobacteria (Table 2). In some of the treated animals skin test reactivity did not develop for as long as 6 weeks after the mycobacteria injection. However, lymphocytes obtained on day 21 during this period of depressed skin test reactivity showed a similar pattern of proliferative responses to tuberculin when compared to cells of animals not receiving 6-mercaptopurine (Table 2).

Some of the animals treated with 6-mercaptopurine developed moderate lymphopenia and marked leukopenia during the study. However, there was no good correlation (correlation coefficient, 0.16) between the degree of depressed skin reactivity and the extent of lymphopenia (Table 2). In addition, the cessation of 6-mercaptopurine treatment was followed by return of lymphocyte counts to normal levels prior to the appearance of tuberculin skin reactivity.

These studies suggest that administration of 6-mercaptopurine did not lead to a qualitative alteration in the proliferative response of guinea pig blood lymphocytes to tuberculin despite concomitant suppression of the development of tuberculin skin reactivity. If the tuberculin-induced lymphocyte proliferation indicates the response of cells which have become "immunologically committed" to this antigen (*7*), several theories can be postulated for the depressed skin reactivity in face of in vitro evidence of immune capability. First, it should be emphasized that the lymphocyte responses compared here occurred in aliquots of 1.0×10^6 lymphocytes each, from either 6-mercaptopurine–treated or untreated animals. Since in vivo lymphopenia of varying degrees was common in the group treated with 6-mercaptopurine, it is conceivable that this substance may suppress the formation in vivo of all lymphoid cells including the subpopulation of cells which would become immunologically committed to tuberculin. Therefore there would be fewer such lymphocytes to recruit into an immune response. Additionally, 6-mercaptopurine therapy might somehow alter in vivo conditions for antigen-responsive lymphocyte activity in a way not reflected by the in vitro assay.

Evidence from other studies suggests possible alternative explanations for the suppressive effect of 6-mercaptopurine on delayed skin test reactivity. The large majority of mononuclear inflammatory cells appearing at the delayed hypersensitivity skin test site appear to be of bone marrow origin and are not specifically sensitized to the antigen in question (*8*). In several studies (*9*), 6-mercaptopurine has been found to exert a profound nonspecific anti-inflammatory effect associated with suppression of the appearance of these mononuclear cells of bone marrow origin in the inflammatory reaction. Therefore, it is possible that the animal with depressed tuberculin skin test reactivity due to 6-mercaptopurine may have sufficient lymphocytes which have become immunologically committed to tuberculin but does not have sufficient numbers of the relatively short-lived cells (*10*) which "nonspecifically" may migrate to and/or be retained at the skin test site in response to some stimulus from a small number of sensitized cells there.

Other experimental observations suggest that a significant portion of the long-lived lymphocyte population persists in the face of 6-mercatopurine administration to become immunologically committed to tuberculin in delayed hypersensitivity response. Delayed skin test reactivity is suppressed only transiently after discontinuation of in vivo administration of 6-mercaptopurine (*1*). In addition, 6-mercaptopurine given for a period prior to but not after the sensitization injection does not lead to suppressed skin test reactivity (*2*).

Since administration of 6-mercaptopurine appears to lead to no qualitative alteration in the response of the sensitized lymphocyte to tuberculin (by at least one criterion), further studies utilizing cell transfer and other in vivo and in vitro techniques may clarify the mechanisms involved.

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Pituitary Serotonin Content: Effects of Melatonin or Deprivation of Water

Abstract. *Relatively high concentrations of serotonin are found in the three regions of the rat pituitary gland. Administration of melatonin causes a selective increase in the serotonin concentration of the pars intermedia; deprivation of water for 5 days causes a selective decrease in the serotonin concentrations of the neural lobe and pars distalis.*

Relatively high concentrations of serotonin have been identified in the pituitary glands of several mammalian species (*1, 2*), especially in the pars intermedia and pars nervosa (*2*). It is not known whether the amine in these regions is localized within pituitary cells, in the terminals of neurons whose cell bodies lie outside the pituitary, or in both. The possible functions of pituitary serotonin also await elucidation. Several observations have led us to examine the effects of administered melatonin on pituitary serotonin concentrations.

These are (i) the decline in the content of melanocyte-stimulating hormone (MSH) in rat pituitaries which occurs soon after animals receive injections of melatonin (*3*), (ii) the changes in brain serotonin contents which follow administration of melatonin (*4*), and (iii) the tendency of circulating ^{3}H-melatonin to be taken up and retained within pituitary tissue (*5*). We have found that melatonin administration causes a significant increase in the serotonin concentration of the pars intermedia without influencing concentrations of serotonin in the other re-